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Clinical Application of the New HPLC Method for Fatty Acid Analysis (2) Effects of a Fat Emulsion on Fatty Acid Compositions in Postoperative Period with Special Reference to C18:1-isomer

TOMONOBU SATO and HIROSHI TANIMURA

Second Department of Surgery, Faculty of Medicine, Kyoto University
(Director: Prof. YORINORI HIKASA)
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Introduction

It was found previously that enteral administration of fat containing linoleic acid as 53% of total fat, induced decreases in eicosapentaenoic acid (EPA) C20:5 and docosahexaenoic acid (DHA) C22:6 contents in the serum phospholipid in postoperative patients[44]. The mechanism of the decreases were speculated to be the suppressed mobilization of EPA and DHA from the liver.

The alternative route to administer fat to patients receiving nutritional support is an intravenous administration of a fat emulsion. The postoperative patients receiving fat emulsions and fat-free infusion were retrospectively reviewed to investigate the effects of a fat emulsion on EPA and DHA contents as well as essential fatty acid (EFA) status and fatty acid composition in the serum phospholipid fraction.

At the same time, the clinical applicability of the new HPLC method[41], especially to the analysis of polyunsaturated fatty acids (PUFA) was discussed, and the *omega*-9/*omega*-6 ratio for assessing EFA status was evaluated.

Materials and Methods

Twenty-four patients undergoing major abdominal surgery were divided into two groups: the Venolipid group (n=16) and the fat-free group (n=8).

The fat-free group: infused regimen consisted of glucose, minerals, and vitamins. No fat emulsion was administered throughout this study in this group.

The Venolipid group: 500 ml of Venolipid a day was administered for three hours via the peripheral vein for seven consecutive days from the 1st through the 7th postoperative day, in

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Present address: Second Department of Surgery, Faculty of Medicine, Kyoto University Sakyo-ku, Kyoto, 606, Japan.

Table 1. Fatty acid composition of fat emulsions

HPLC method (weight %)

	Lecithin			Triglyceride		
	soy bean	egg yolk		soy bean		
	Venolipid	Intralipos	Intrafat	Venolipid	Intralipos	Intrafat
12:0	9.34	1.58	0.45	1.86	tr.	0.21
14:0	tr.	tr.	tr.	N.D.	N.D.	N.D.
16:0	13.70	31.07	23.64	11.84	12.01	12.16
16:1	N.D.	0.78	0.27	N.D.	N.D.	N.D.
18:0	24.48	12.80	8.86	3.92	2.92	3.87
18:1	12.93	28.22	25.81	22.43	22.88	22.43
18:1-isomer	35.29	N.D.	0.21	N.D.	N.D.	N.D.
18:2	3.92	14.90	32.06	52.18	54.19	51.74
18:3	tr.	0.65	4.26	6.10	8.00	4.55
20:3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
20:4	0.34	3.01	1.19	N.D.	N.D.	N.D.
20:5	N.D.	N.D.	N.D.	N.D.	N.D.	0.28
22:6	tr.	6.59	3.25	1.47	tr.	2.53

N.D.: not detected, tr.: trace

addition to glucose solution containing vitamins and minerals.

All the patients recovered without serious postoperative complications and oral intake was forbidden except for only sips of water or ice until the 8th postoperative day, thereafter, they were given a liquid diet and subsequently normal diet by the 15th postoperative day. The volume of infusion was 2500 ml/day, and the calories provided was around 1200 Cal/day in both groups. No amino acid solution was given throughout the study in either group.

The administered fat emulsion, Venolipid[47], is newly developed by MORISHITA Pharmaceutical Co. Ltd. (Shiga, Japan). It consists of soy bean triglyceride (50 g/500 ml), soy bean lecithin (6 g/500 ml) and additional glycerol (12.5 g/500 ml) to make it isoosmolar. The triglyceride contains 52.18% linoleic acid, nearly the same level present in other commercially available fat emulsions employing soy bean oil (Table 1), therefore, about 20% of the total calories was supolied

Table 2. Composition and linoleic acid content in fat emulsions and chemically defined diets

	Venolipid 500 ml	Intralipos 500 ml	Elental	Ensure
Triglyceride	Soybean oil (51%)	Soybean oil (54%)	Soybean oil	Corn oil
Phospholipid	Soybean oil (6 g)	Yolk egg (6 g)	(÷)	(÷)
Linoleic acid in total calories (%)	19.9	19.9	0.9	18.9

*: 1200 Kcal supplied a day

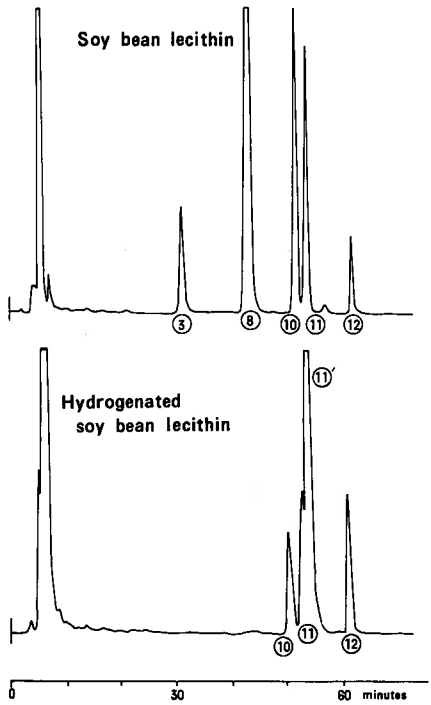


Fig. 1.

	% composition	
fatty acids	before	after
③ $C_{18:3}$	7.13	N.D.
⑧ $C_{18:2}$	56.45	tr.
⑩ $C_{16:0}$	17.56	12.95
⑪ $C_{18:1}$	14.66	10.42
⑪′ $C_{18:1}$-isomer	N.D.	63.70
⑫ $C_{18:0}$	3.46	12.93
unidentified	0.74	0
total	100	100

as linoleic acid in the Venolipid group. Linoleic acid content in the present study and the previous study is shown in Table 2.

Its characteristic feature is its phospholipid, which is added to emulsify the oil. Venolipid contains soy bean lecithin instead of the yolk egg lecithin used in other commercially available fat emulsions. Moreover, it contains unusual fatty acids, C18:1-isomer, derived from hydrogenation of soy bean lecithin (Fig. 1).

Blood was withdrawn at 8:00 a.m. from the superficial antecubital vein before the study, on the 8th and 15th postoperative day. After centrifugation, 0.1 ml of serum was subjected to lipid extraction, fractionation by TLC, hydrolysis and derivatization for fluorescent detection.

Fatty acid analysis was carried out by the new HPLC method devised by the author. The details of pretreatment procedures were described in the previous report[41]. Fatty acid compositions in the serum phospholipid fractions between the two groups, one receiving Venolipid and another no fat supplement, were compared.

Values are given as the mean $\pm$ SE, and differences among the values before, on the 8th day and 15th day were tested for statistical difference by Student's "t" test.

Results

Individual fatty acid levels in the serum phospholipid fraction from both groups are given in Tables 3-5. The mean values for each fatty acid are shown in Table 6.

Table 3. Fatty acid levels in the serum phospholipid fraction before

Patient	1			2			3			4		
	I	II	III	I	II	III	I	II	III	I	II	III
12:0	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
14:0	1.45	0.38	0.47	0.87	0.44	1.05	0.48	0.38	0.65	2.09	0.76	0.53
16:0	38.40	29.08	37.13	53.60	43.47	48.03	18.39	52.07	44.31	53.86	47.75	43.47
16:1	3.97	1.18	1.78	6.68	2.09	4.30	4.15	1.81	1.54	3.12	1.55	1.22
18:0	16.95	22.40	17.53	28.57	31.20	27.59	7.71	34.85	31.62	17.20	15.80	11.82
18:1	16.22	10.51	14.65	25.15	16.26	23.66	29.86	20.02	16.34	25.26	19.16	23.68
18:1-isomer	N.D.	31.79	N.D.	0	47.88	0	N.D.	59.28	58.45*	N.D.	60.65	58.23
18:2	17.36	20.29	22.73	20.38	27.23	26.98	33.93	31.71	25.90	35.16	34.28	31.77
18:3	tr.	0.20	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:3	2.89	1.19	2.14	3.19	1.73	3.33	3.26	1.70	1.29	2.88	1.52	1.71
20:4	9.83	6.33	13.00	11.92	7.45	12.32	20.85	11.75	8.47	21.69	14.36	13.21
20:5	2.50	0.86	2.31	3.68	2.55	3.26	2.27	1.11	0.79	5.07	1.50	1.41
22:6	11.64	6.59	9.27	26.10	11.60	17.68	20.00	10.80	8.08	14.90	9.70	9.82
unidentified	2.80	0.20	2.00	1.90	0.10	1.80	2.10	0.50	0.60	5.80	0.10	0
total	124	131	123	182	192	170	143	226	198	187	207.1	197

N.D.: not detected, tr.: trace
*Blood sample was taken on the 12th day.

Table 4. Fatty acid levels in the serum phospholipid fraction before

Patient	9			10			11			12		
	I	II	III	I	II	III	I	II	III	I	II	III
12:0	0	tr.	0.03	tr.	tr.	0.80	0	tr.	tr.	0.59	0.16	tr.
14:0	0	tr.	0.06	0.26	0.34	0.34	tr.	tr.	tr.	1.18	0.47	0.15
16:0	52.64	57.31	45.60	42.67	43.73	41.73	20.15	39.30	33.84	55.99	42.10	44.72
16:1	2.14	1.20	1.48	2.83	1.47	1.35	2.88	1.39	0.65	2.66	0.52	0.31
18:0	18.39	12.09	17.90	15.44	25.30	20.33	5.44	31.81	16.82	19.45	14.72	15.59
18:1	22.36	26.77	19.56	15.79	10.88	21.25	25.29	12.21	17.92	23.38	14.62	29.29
18:1-isomer	N.D.	28.09	3.53	N.D.	26.26	N.D.	N.D.	47.09	N.D.	N.D.	60.76	N.D.
18:2	30.32	37.77	25.76	16.45	32.51	29.63	46.26	24.44	23.34	27.05	29.27	31.45
18:3	tr.	tr.	tr.	tr.	tr.	tr.	0.01	tr.	tr.	tr.	tr.	tr.
20:3	2.36	2.37	3.64	2.00	1.25	1.74	2.34	0.68	1.64	2.04	1.59	4.50
20:4	13.29	18.09	16.08	10.13	7.01	7.25	19.21	9.32	7.61	7.61	7.89	9.10
20:5	2.08	1.94	3.18	3.04	1.97	1.35	4.66	0.97	1.45	0.71	1.43	1.64
22:6	8.90	10.35	10.76	11.23	11.10	11.32	9.62	3.67	4.38	6.33	7.31	8.66
unidentified	1.50	0	1.40	0	0.10	0.10	1.10	0.10	0.30	1.00	0.20	0.60
total	154.0	196.0	149.0	120.0	161.9	137.2	137	171	108	148	181	146

N.D.: not detected, tr.: trace
*Blood sample was taken on the 12th day.

(I), on the 8th day (II) and the 15th day (III) in the Venolipid group

(mg/dl)

5			6			7			8		
I	II	III	I	II	III	I	II	III	I	II	III
tr.	tr.	tr.	1.58	1.11	tr.	4.86	0.58	—	0.88	tr.	tr.
0.56	0.60	0.82	2.27	0.86	0.74	1.29	tr.	—	0.59	tr.	tr.
41.22	43.09	52.21	23.36	46.44	62.47	23.79	30.91	—	49.45	44.40	40.90
0.72	0.81	0.51	4.72	0.61	2.76	4.68	2.10	—	2.70	1.74	1.54
19.37	23.93	25.53	8.11	27.69	24.86	8.55	18.55	—	23.42	27.44	23.09
22.07	17.29	29.12	27.36	15.15	21.33	25.15	21.67	—	20.44	14.66	20.32
N.D.	38.53	0	N.D.	45.41	N.D.	N.D.	45.90	—	N.D.	40.04	N.D.
25.92	29.84	36.71	52.64	29.96	30.54	45.41	32.97	—	22.37	30.79	33.14
tr.	tr.	tr.	1.60	tr.	tr.	tr.	tr.	—	tr.	tr.	tr.
2.55	2.53	3.30	2.48	1.57	2.85	3.35	1.37	—	4.54	2.46	4.88
7.76	9.17	14.12	11.68	13.96	19.08	19.05	9.87	—	11.53	6.80	13.51
0.56	0.31	1.40	9.73	1.61	1.53	3.59	1.49	—	2.96	1.76	3.25
4.45	6.17	7.34	13.12	10.56	15.81	16.05	9.05	—	15.00	9.23	12.21
1.80	0.10	0	1.30	0.10	2.00	0.40	0.50	—	1.10	1.70	0.10
127	172.3	171.1	160	195	184	156	175	—	155	181	152.9

(I), on the 8th day (II) and the 15th day (III) in the Venolipid group

(mg/dl)

13			14			15			16		
I	II	III	I	II	III	I	II	III	I	II	III
tr.	tr.	tr.	0.53	0	0.07	0.46	tr.	—	0	tr.	tr.
0.66	0.29	0.68	tr.	tr.	0.21	0.73	0.40	—	tr.	tr.	tr.
56.76	49.21	48.07	44.81	43.33	33.04	47.53	57.48	—	39.92	36.47	48.65
4.38	3.11	4.31	0.29	0.21	0.72	0.95	0.69	—	0.95	0.88	2.13
16.52	14.18	14.32	16.44	28.33	21.78	18.51	37.23	—	15.84	25.88	23.62
34.70	23.72	44.90	18.65	12.62	9.69	22.41	17.52	—	25.00	10.81	23.09
N.D.	46.50	N.D.	N.D.	46.30	34.92*	N.D.	50.57	—	N.D.	41.16	N.D.
53.58	62.43	62.86	21.63	24.74	20.86	15.55	32.77	—	28.35	25.28	35.27
tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	—	tr.	tr.	tr.
2.62	1.57	2.42	2.41	1.64	1.60	2.40	0.73	—	3.43	1.79	2.80
9.07	9.16	12.92	8.98	7.73	9.69	10.00	11.94	—	10.43	8.58	12.62
0.93	1.34	1.41	1.09	0.68	0.70	1.62	1.40	—	3.78	1.37	1.94
13.14	12.45	14.61	10.16	8.61	6.35	11.65	11.90	—	22.29	5.74	8.43
1.60	0	0.50	0	0	0.10	3.20	0.40	—	0.40	0	1.00
192	224	207	125	174.2	139.7	135	223	—	150	158	159

Table 5. Fatty acid levels in the serum phospholipid fraction before

Patient	1			2			3			4		
	I	II	IV	I	II	III	I	II	III	I	II	III
12:0	—	tr.	tr.	tr.	0.06	tr.	tr.	tr.	0.17	tr.	N.D.	tr.
14:0	tr.	0.02	0.14	tr.	1.27	0.28	0.24	tr.	0.43	0.49	tr.	tr.
16:0	46.66	33.39	37.74	53.10	52.20	47.46	38.74	43.89	41.54	49.74	49.76	40.91
16:1	0.72	0.12	1.27	1.05	2.37	2.34	1.43	1.16	1.14	4.28	4.09	1.57
18:0	22.16	18.67	24.25	15.20	15.84	13.01	18.18	19.30	18.62	22.89	18.88	18.38
18:1	15.99	18.41	20.87	21.95	19.17	18.16	16.56	15.99	17.69	18.71	14.42	21.03
18:2	22.96	20.7	23.34	31.90	25.97	29.38	22.22	25.48	25.18	28.22	23.64	26.60
18:3	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:3	0.78	1.89	1.84	1.04	1.98	2.12	3.13	2.30	2.18	2.48	1.71	6.31
20:4	11.19	9.52	12.49	10.09	16.66	12.11	7.48	8.80	8.97	14.94	16.19	1.87
20:5	4.78	2.28	2.41	2.75	2.31	1.95	1.13	1.26	0.87	2.10	2.96	0.54
22:6	14.55	12.48	14.95	21.89	15.94	13.94	9.85	9.46	7.86	19.34	14.03	10.21
unidentified	0.10	0.50	0.20	3.00	3.60	1.20	2.00	0.20	0.10	0.80	0.30	0.60
total	139.9	118	139.5	162	157.4	142	118	127.8	124.8	164	146	128

N.D.. not detected, tr.: trace

The changes in representative fatty acids were shown in Figs. 2 to 5.

1. *Omega*-6 fatty acids (Fig. 2)

No significant change was observed in linoleic acid content in the Venolipid group throughout this study. However, in the fat-free group a decrease in linoleic acid on the 15th day from the initial level of 28.53±2.49 mg/dl to 21.07±0.90 mg/dl was observed ($p<0.05$).

In the Venolipid group, arachidonic acid decreased from the initial level of 12.69±1.19 mg/dl to 9.96±0.82 mg/dl on the 8th day, but rose to 11.37±1.11 mg/dl on the 15th day, although these changes were not statistically significant.

In the fat-free group a decrease in arachidonic acid was observed on the 15th day, from 13.28±1.21 mg/dl to 9.99±1.51 mg/dl ($p<0.05$), but no significant decrease was observed on the 8th day compared to the initial level.

2. *Omega*-9 fatty acids (Fig. 3)

In the Venolipid group, palmitoleic acid also decreased from the initial level of 2.99±0.44 mg/dl to 1.34±0.18 mg/dl on the 8th day ($p<0.05$) and still remained low (1.76±0.34 mg/dl) on the 15th day ($p<0.05$). No changes were observed in palmitoleic acid as in oleic acid in the fat-free group.

Oleic acid decreased from 23.69±1.20 mg/dl to 16.49±1.20 mg/dl ($p<0.05$) in the Venolipid group on the 8th day but returned to the initial level on the 15th day. No changes were observed in the fat-free group.

In the Venolipid group, a marked decrease in eicosatrienoic acid was also observed on the 8th day from 2.80±0.64 mg/dl to 1.61±0.13 mg/dl ($p<0.01$). In contrast, in the fat-free group

(I), on the 8th day (II) and the 15th day (III) in the fat-free group

(mg/dl)

5			6			7			8		
I	II	III	I	II	III	I	II	III	I	II	III
0.19	0.38	0.38	0.33	0.35	1.34	2.58	tr.	tr.	0.41	tr.	tr.
0.28	0.90	0.39	0.60	0.09	0.70	tr.	tr.	tr.	0.20	tr.	tr.
49.39	44.38	52.63	54.39	50.56	39.56	67.80	67.67	66.83	46.16	30.25	34.09
4.02	4.48	4.50	4.32	2.35	3.38	3.49	3.63	2.81	1.84	0.97	1.71
20.53	26.19	23.58	25.41	18.17	16.02	27.01	21.44	26.73	13.35	16.11	22.18
20.19	24.17	25.03	26.79	22.87	22.37	22.02	16.32	23.66	13.65	18.10	19.78
24.92	28.77	28.35	33.16	28.09	26.79	42.41	34.73	41.44	22.43	26.30	23.06
tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
2.40	3.50	3.68	3.11	1.60	1.82	1.77	1.84	2.10	0.72	1.60	2.77
14.40	8.50	13.71	17.29	7.03	14.37	16.83	22.54	10.51	14.02	3.01	5.87
4.41	2.27	2.98	1.57	1.39	0.94	5.20	6.05	2.69	1.99	0.88	0.93
20.53	12.13	14.65	14.55	11.40	9.91	23.17	22.99	20.73	11.91	5.69	8.22
1.10	4.30	1.20	1.50	1.30	0.80	0.70	0.30	0.50	0.40	0.10	0.40
162.4	160	171.1	183	145.2	138	213	197.5	198	127.1	103	119

no change was observed on the 8th day. On the 15th day, the level rose to the initial level in the Venolipid group, whereas, in the fat-free group, the level (2.85 ± 0.54 mg/dl) was higher than the initial level of 1.93 ± 0.35 mg/dl ($p<0.05$), although no increase was observed on the 8th day.

Table 6. Fatty acid levels in the serum phospholipid fraction before (I), on the 8th day (II) and the 15th day (III), in the Venolipid group and the fat-free group.

(mg/dl)

	Venolipid group			fat-free group		
	I (n=16)	II (n=16)	III (n=14)	I (n=8)	II (n=8)	III (n=8)
12:0	0.55±0.30	0.12±0.08	0.06±0.06	0.04±0.31	0.10±0.06	0.24±0.16
14:0	0.78±0.18	0.31±0.07	0.41±0.09	0.23±0.08	0.29±0.18	0.24±0.09
16:0	41.43±3.31	44.13±1.99	44.58±2.03	50.75±2.97	46.51±3.86	45.10±3.71
16:1	2.99±0.44	1.34±0.18	1.76±0.34	2.64±0.54	2.40±0.56	2.34±0.41
18:0	15.37±1.57	24.46±1.91	20.89±1.48	20.59±1.69	19.33±1.17	20.35±1.63
18:1	23.69±1.20	16.49±1.20	22.49±2.22	19.48±1.47	18.68±1.19	21.07±0.90
18:1-isomers	N.D.	44.76±2.61	11.03±5.50	N.D.	N.D.	N.D.
18:2	30.77±3.17	31.64±2.32	31.21±2.75	28.53±2.49	26.71±1.45	21.07±0.90
18:3	0.10±0.01	0.10±0.01	N.D.	N.D.	tr.	tr.
20:3	2.80±0.16	1.61±0.13	2.50±0.36	1.93±0.35	2.05±0.22	2.85±0.54
20:4	12.69±1.19	9.96±0.82	11.37±1.11	13.28±1.21	11.53±2.25	9.99±1.51
20:5	3.02±0.56	1.39±0.14	1.83±0.23	2.99±0.56	2.43±0.57	1.66±0.34
22:6	13.41±1.42	9.05±0.63	10.34±0.99	16.97±1.74	13.02±1.79	12.56±1.54
total	149.8 ±5.6	185.5 ±6.5	160.1 ±7.8	158.7 ±10.9	144.4 ±10.3	145.1 ±9.4

mean±SE, N.D.: not detected, tr.: trace

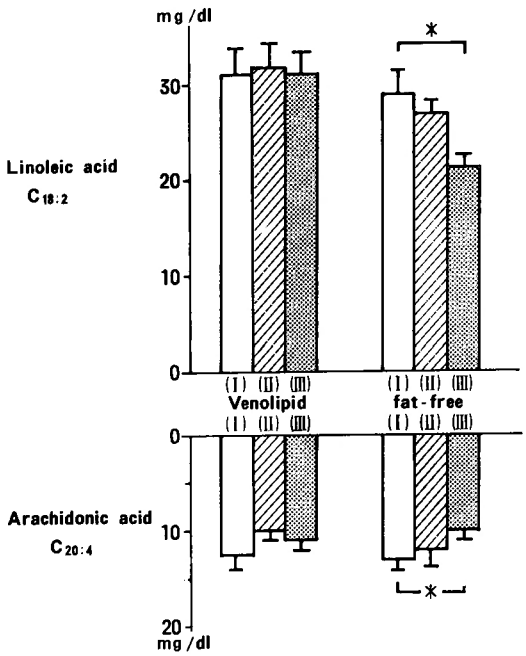


Fig. 2. Fatty acid levels in the serum phospholipid fraction before (I), the 8th day (II) and the 15th day (III), in the Venolipid group and the fat-free group. mean±SE, *: $p<0.05$

3. T/t ratio (Figs. 4 and 5, Table 7)

Changes in the t/t ratio in each patient are shown in Fig. 4 and the mean value in Table 7.

In the Venolipid group, all patients showed decreases in the t/t ratio on the 8th day, and the values on the 15th day were still lower than the initial values. On the other hand, in the fat-free group, 6 of 8 patients showed increased values on the 15th day, compared to the initial values. Even on the 15th day the t/t ratios tended to remain lower than the initial values in the Venolipid group. In contrast, in the fat-free group the t/t ratio tended to be elevated and it was still higher on the 15th day than the initial values.

The mean values of t/t ratios in both groups are shown in the left half of Fig. 5. In the Venolipid group a decline in the ratio was observed from the initial value of 0.24 ± 0.02 to 0.17 ± 0.02 on the 8th day ($p<0.05$), and returned to 0.23 ± 0.03 on the 15th day. On the contrary, in the fat-free group a rose in the ratio from 0.16 ± 0.04 to 0.24 ± 0.06 on the 8th day and to 0.23 ± 0.04 on the 15th day was observed, but they were not statistically significant.

4. *Omega*-9/*omega*-6 ratio (Figs. 5 and 6, Table 7)

The ratio for each case is shown in Fig. 6 and the mean value was given in Table 7 and Fig. 5. In the Venolipid group, all patients except one showed marked decreases on the 8th day. In the fat-free group, 5 of 8 patients showed slight increases in this ratio on the 15th day, compared to the initial values.

The mean value was shown in the right half of Fig. 5. In the Venolipid group, a significant

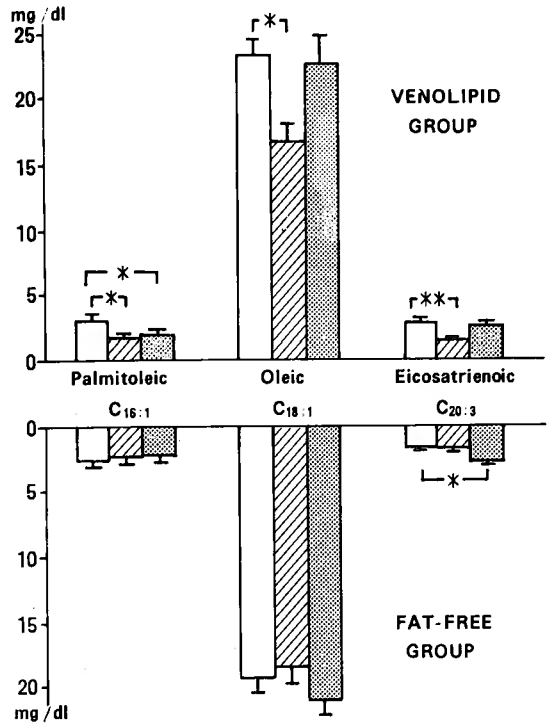


Fig. 3. Fatty acid levels in the serum phospholipid fraction before (I), the 8th (II) and the 15th (III) day, in the Venolipid group and the fat-free group
mean ±SE, *: $p<0.05$, **: $p<0.01$

decrease on the 8th day from the initial level of 0.74±0.05 to 0.47±0.02 ($p<0.05$) was observed but the level rose to 0.62±0.03 (not significantly different from the initial level) on the 15th day. In the fat-free group, a slight elevation was observed on the 8th day, from 0.55±0.04 to 0.62±0.06,

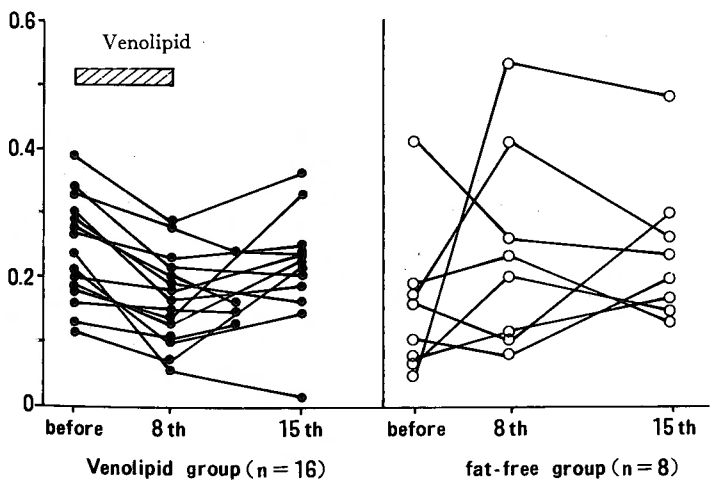


Fig. 4. Triene/tetraene ratio before, on the 8th day and the 15th day in the Venolipid and the fat-free group

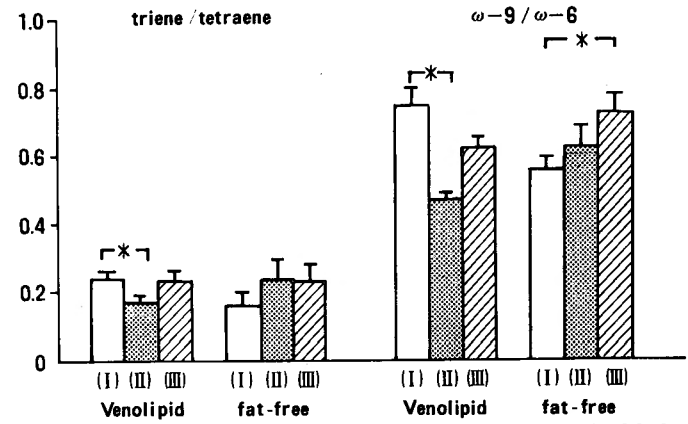


Fig. 5. Ratios of triene/tetraene and ω-9/ω-6 before (blank bars) on the 8th day (dotted bars) and on the 15th day (shaded bars)
mean±SE, *: $p<0.05$

Table 7. The ratios of triene/tetraene and ω-9/ω-6 in the Venolipid and fat-free groups

	Venolipid group		fat-free group	
	t/t	ω-9/ω-6	t/t	ω-9/ω-6
I	0.34±0.02 (n=16)	0.74±0.05 (n=16)	0.16±0.04 (n=8)	0.55±0.04 (n=8)
II	0.17±0.02 (n=16)	0.47±0.02 (n=16)	0.24±0.06 (n=8)	0.62±0.06 (n=8)
III	0.23±0.03 (n=14)	0.62±0.03 (n=14)	0.23±0.04 (n=8)	0.71±0.06 (n=8)

mean±SE
I: before, II: on the 8th day, III: on the 15th day

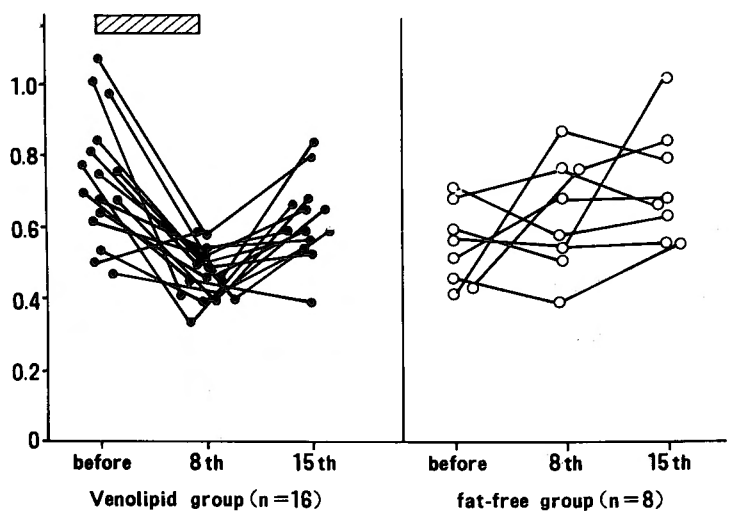


Fig. 6. ω-9/ω-6 ratio before, on the 8th day and the 15th day in the Venolipid group and the fat-free group

however, a significant increase to 0.71 ± 0.06 ($p<0.05$) was seen on the 15th day.

5. *Omega*-3 fatty acids (Fig. 7)

Decreases in EPA and DHA were also found in both groups.

In the Venolipid group EPA decreased from the initial level of 3.02 ± 0.56 mg/dl to 1.39 ± 0.14 mg/dl on the 8th day ($p<0.05$) and still remained low (1.83 ± 0.23 mg/dl) on the 15th day ($p<0.05$). Although no significant decrease was observed on the 8th day, a moderate decline was noted on the 15th day from the initial level of 2.99 ± 0.56 mg/dl to 1.66 ± 0.34 mg/dl ($p<0.05$) in the fat-free group.

In the Venolipid group, DHA also decreased from 13.41 ± 1.42 mg/dl to 9.05 ± 0.63 mg/dl ($p<0.05$) on the 8th day, and 10.34 ± 0.99 mg/dl ($p<0.05$) on the 15th day. In the fat-free group a moderate decrease in DHA was observed on the 15th day from the initial level of 16.97 ± 1.74 mg/dl to 12.56 ± 1.54 mg/dl ($p<0.05$), although no significant decrease was seen on the 8th day.

6. C18:1-isomer

This fatty acid is not detected in appreciable amounts normally. No change was observed in the fat-free group.

On the contrary, in the Venolipid group, C18:1-isomer was detected at a high level of 44.76 ± 2.61 mg/dl on the 8th day (the day after discontinuation of the administration). How-

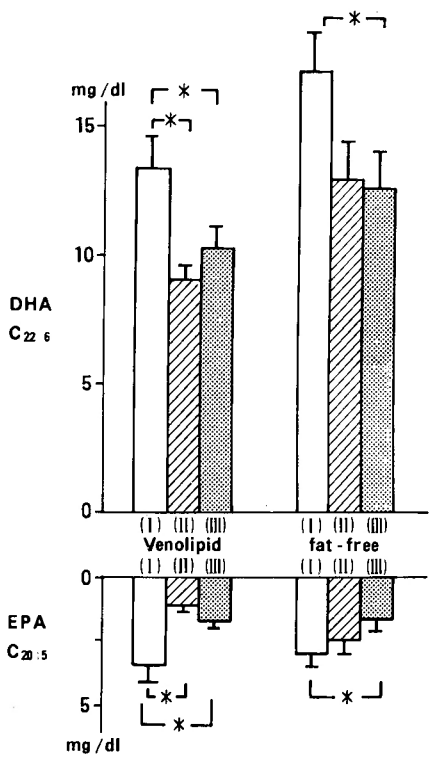


Fig. 7. Fatty acid levels in the serum phospholipid fraction before (I), the 8th (II) and the 15th (III) day, in the Venolipid group and the fat-free group.
mean $\pm$SE, *: $p<0.05$

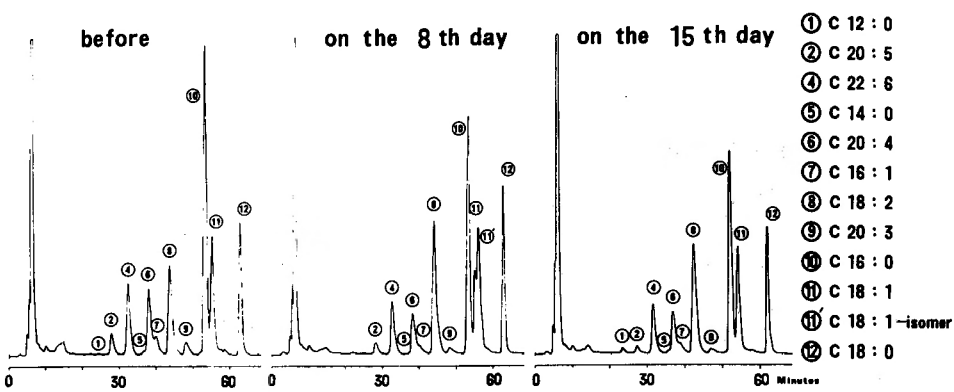


Fig. 8. HPLC chromatogram of the serum phospholipid from a 32 y.o. male receiving Venolipid for 7 days

ever, C18:1-isomer was not detected on the 15th day (7 days after discontinuation of Venolipid) as shown in Fig. 8. The mean level of C18:1-isomer on the 15th day was 11.03±5.50 mg/dl, due to the high content in two patients whose blood samples were taken on the 12th day. In patients 3 and 14, C18:1-isomer still remained at 58.45 and 34.92 mg/dl, respectively. However, no detectable amount of C18:1-isomer was found in sera from the other patients.

Discussion

Fat emulsions including Venolipid contain linoleic acid as 50% of the triglyceride prepared from soy bean oils.

The fatty acid composition of the phospholipids in fat emulsions is different from that of triglyceride. As the phospholipid of Venolipid is prepared from soy bean, its fatty acid composition is also different from those of other fat emulsions prepared from yolk eggs.

Linoleic acid is supplied as triglyceride by the administration of Venolipid, and linoleic acid in the serum triglyceride increased markedly from 24.62±2.45% to 35.14±2.53% (on the 8th day) but returned to 25.55±2.32% (on the 15th day). Although an increase in linoleic acid in the serum phospholipid was also expected, neither linoleic acid nor arachidonic acid increased in the serum phospholipid in the Venolipid group.

The reason for the absence of an increase in linoleic acid in the serum phospholipid, in the presence of an increase in linoleic acid in the serum triglyceride, is not clear. As transfer of fatty acids from one fraction into other fractions occurs in the liver[4,35], it is speculated that some mechanism inhibits linoleic acid in triglyceride from being transferred into phospholipid. Venolipid contains large amounts of C18:1-isomer, namely, 35.29% of its phospholipid. This isomer might have prevented the transfer of linoleic acid from serum triglyceride into phospholipid. Moreover, it is known that C18:1-isomer inhibits the conversion of linoleic acid to arachidonic acid[26,27,34].

It was also found that if fatty acid analysis is carried out in total lipids or triglyceride fraction, an increase in linoleic acid would lead clinicians to misjudge the EFA status, despite of no increase

in linoleic acid or arachidonic acid in the serum phospholipid. Judging from the discrepancy in EFA content between the serum triglyceride and phospholipid fractions, evaluation of EFA status should be done not for total lipids but for individual lipid fractions, especially the phospholipid fraction, because of its physiological significance[1,3,5].

In the Venolipid group, the t/t ratio decreased in all patients and its level on the 15th day still remained lower than the initial levels in 9 of 16 patients. On the contrary, in the fat-free group, although no definite tendency was found in individual patients, increases in the t/t ratios observed on the 8th day remained at the same level on the 15th day. However, they were not statistically significant. A marked decrease in the t/t ratio in the Venolipid group contrasted strikingly to that in the fat-free group, although no difference was observed in linoleic acid or arachidonic acid content on the 8th day.

The decrease in the t/t ratio was due to a decrease in eicosatrienoic acid in the Venolipid group. It is well known that linoleic acid and arachidonic acid suppress *omega*-9 fatty acid synthesis[16,46]. However, no increase in these two fatty acids was observed, thus they could not have been responsible for the suppressed eicosatrienoic acid synthesis. However, the intravenous administration of Venolipid suppressed eicosatrienoic acid synthesis and induced a decrease in the t/t ratio.

In discussing the EFA status, the authors proposed the evaluation of the *omega*-9/*omega*-6 ratio together with each sum of *omega*-9 and -6 fatty acids[44]. Comparing the t/t ratio with the *omega*-9/*omega*-6 ratio in the fat-free group, no significant difference was observed in the former, on the other hand, a significant increase was observed in the latter on the 15th day. This increase was due to a decrease in *omega*-6 fatty acids and an increase in *omega*-9 fatty acids, although neither was statistically significant. On the contrary, a decrease in the *omega*-9/*omega*-6 ratio in the Venolipid group was due to a significant decrease ($p<0.05$) in *omega*-9 fatty acids.

In the early postoperative period (until 8th day), patients undergoing major abdominal surgery can be managed with no fat supplement, without developing biochemical EFA deficiency. However, biochemical EFA deficiency was observed on the recovery period (8th to 15th day), judging from the *omega*-9/*omega*-6 ratio and the sum of *omega*-6 fatty acids, although the deficiency was not apparent only from the t/t ratio.

These observations of significant decreases in all of *omega*-9 fatty acids, as well as the t/t and *omega*-9/*omega*-6 ratios during the administration of Venolipid suggest the applicability of the intravenous administration of a fat emulsion for prevention of EFA deficiency even during a short-term period of seven days. Moreover, the effect of the administration on EFA status continued for seven days after the discontinuation of the administration.

WENE et al.[49] demonstrated that biochemical EFA deficiency occurs even in healthy volunteers receiving a fat-free regimen as early as day 4. However, in our study no patient in the fat-free group showed a EFA deficiency pattern in the serum phospholipid for seven days. The primary cause for the difference might be that caloric supply was higher in the WENE's study than in our study, because EFA requirement increases with the provided calories[9,17]. Second, as dietary habits have a marked effect on the fatty acid composition in blood and tissues, Japanese, who

consume much vegetable oils and fish oils than the western people, may response differently to the fat-free treatment. EFA requirement increased when the supplied calories are large and large amounts of fat excluding EFA is supplemented[9,17]. Moreover, infants and children should not be managed by fat-free hyperalimentation[7,22], because EFA is necessary for normal mental development and body growth, thus the young require a higher content of EFA than do adults.

Moreover, our new HPLC method permitted the investigation of changes in EPA and DHA during the early postoperative and recovery period. Special interest in these fatty acids has been increasing recently, since their important physiologic roles were partly elucidated[6,10,38,39,40,45,48]. However, the lack of rapid and reliable analytical methods to determine EPA and DHA limits the study of their metabolism in human subjects.

Both EPA and DHA must be supplied exogenously, because no appreciable synthetic pathway has been confirmed in human subjects.

EPA and DHA are found at high levels in the liver[25], testis and heart, but only small amounts in rat adipose tissue[36]. Less than 0.32% of EPA and 2.0% of DHA was detected in abdominal subcutaneous adipose tissue obtained at laparotomy by our HPLC method (unpublished data).

No report has described the changes in EPA and DHA contents in human serum in the early postoperative period. We found that the Venolipid group exhibited significant decreases in EPA and DHA compared to the fat-free group. During our study no supplement of EPA of DHA was given, therefore, they had to be supplied endogenously, namely, mobilization from the liver. Marked decreases in EPA and DHA were observed during the administration of Venolipid (until the 7th day), and the levels of EPA and DHA began to increase following discontinuation of the administration. It is reasonable to speculate that the administration of fat emulsions suppresses fatty acid mobilization from the liver.

On the other hand, in the fat-free group mobilization of these fatty acids occurred but not enough to maintain their initial levels. Thus, no decrease was observed until the 15th day when effect of no exogenous supplementation of these fatty acids appeared.

These observations suggest that the administration of a fat emulsion affects EPA and DHA metabolism in human subjects. However, it is not clear whether these effects were due to fat contents or linoleic acid itself. Although the precise explanation remains to be elucidated, it is probable that the administered linoleic acid affects EPA and DHA metabolism in human subjects. It is well known that a competitive interaction between the three families of PUFA occurs, such that *omega*-3 acids suppress the metabolism of *omega*-6 acids, *omega*-6 acids suppress the metabolism of *omega*-3 acids less strongly, and both of these acids suppress the formation of long-chain *omega*-9 acids[9,17].

From these results, it is necessary to pay special attention to EPA and DHA, which have been neglected in previous papers due to lack of anlytical instruments for their rapid and accurate determination. Our new HPLC method would contribute to the investigation of these fatty acids.

As discussed previously, enterally administered linoleic acid increases linoleic acid in the serum phospholipid fraction[44].

If there had been any differences in the administered linoleic acid content or supplied calories

between the intravenously and enterally administered groups, there should be differences in fatty acid metabolisms such as transportation, consumption, utilization, synthesis of other fatty acids, and storage. But no such differences were detected.

Therefore, it was concluded that the observed difference in linoleic acid content in the serum phospholipid was caused by the difference in modes of administration.

Enterally administered linoleic acid as triglyceride is hydrolyzed by pancreatic lipase with the aid of bile acids and absorbed in the intestinal mucosa. It is reincorporated into triglyceride and bound with carrier protein to form chylomicrons and transferred to the liver via the portal vein.

On the contrary, intravenously administered linoleic acid in triglyceride is carried directly to the liver via peripheral blood. Thus, there are differences between the intravenous and enteral administrations in the modes of transportation, namely, chylomicron versus triglyceride, and via portal versus peripheral blood. These differences may cause differences in fatty acid metabolism, linoleic and eicosatrienoic acid contents in serum phospholipid, between the intravenously and enterally administered groups.

The intravenous administration of a fat emulsion (linoleic acid) also suppressed the increase in eicosatrienoic acid in human serum, whereas, no decrease was observed in a short-term trial of Ensure for seven days (enteral administration of fat)[44].

We demonstrated the difference in fatty acid metabolism between intravenous and enteral administration, in a short-term trial.

However, it must be added that enterally administered fat might suppress eicosatrienoic acid synthesis in a longer-period trial, although a slight increase in eicosatrienoic acid was found in spite of an increase in linoleic acid in the Eusure group.

By the new HPLC method an unidentified peak (peak 11') was found just after the oleic acid C18:1 (peak 11) in the phospholipid of Venolipid[42,43]. This peak could not be separated by the routine gas liquid chromatography. Peak 11' is also confirmed after oleic acid (peak 11) in the HPLC chromatogram of hydrogenated soy bean lecithin. Thus, the unidentified peak should be produced by hydrogenation of soy bean lecithin before being mixed with triglyceride, in order to give more stability to the fat emulsion.

Hydrogenation induces not only reduction of the number of double bonds but also migration of double bonds along the carbon chain, and positional and geometrical (*cis-, trans-*) isomers. Moreover, unsaturated fatty acids content were decreased after hydrogenation compared to those before hydrogenation.

Most of the naturally occurring unsaturated fatty acids have double bonds of the *cis* configuration. The most abundant dietary *cis* monoenoic fatty acid is oleic (*cis* 9–18:1). The best known monounsaturated fatty acid with *trans* configuration is vaccenic acid, *trans* 11-C18:1, which is found in microorganisms and in the fat of ruminants[37].

In human subjects, oleic acid is the only naturally occurring C18:1 fatty acid. Retention time of vaccenic acid, one of the isomers of C18:1, is identical to that of the peak 11' Thus, the fatty acid of peak 11' was considered to be C18:1 isomers. Isomers of C18:1 are not

homogeneous and consist of various mixtures which are different in the double bond position and *cis*-, *trans*-configuration. Our HPLC method separates only one peak (peak 11') from oleic acid (peak 11), thus, this peak is composed of mixtures of isomers. Complete separation between peaks 11 and 11' is achieved by modification of gradient technique, requiring a longer analysis time (87 minutes).

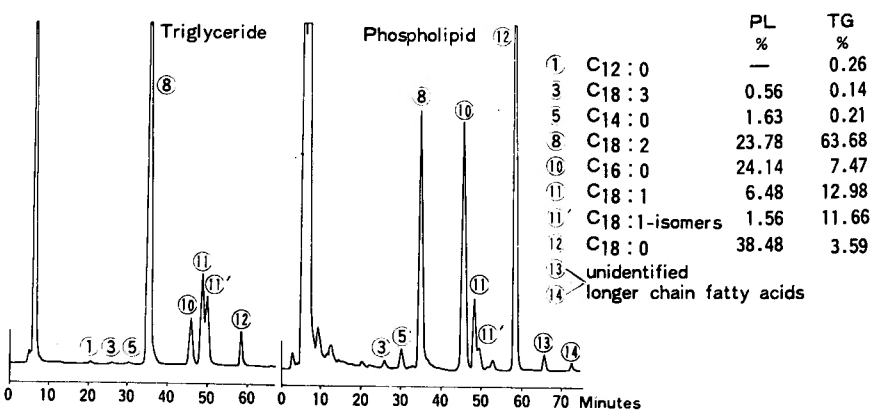
The C18:1-isomer remained at a level of 44.76±2.61 mg/dl, the day after the termination of administration (on the 8th day), but disappeared seven days later in all patients, and was detected only in the serum phospholipid but not in the serum triglyceride. This finding also implies that there is no interconversion of this fatty acid from phospholipid to triglyceride. This might be true for all fatty acids, but no detail report is available. However, the conversion of fatty acid from triglyceride to phospholipid was confirmed by the study using 14C-labeled fatty acids[14].

Unnatural C18:1-isomers have increased in the environment. Many works have been done on octadecenoic C18:1-isomers occurring in margarines[2,21,23,31] and fish oils which is hydrogenated to improve stability. For example, one type of soft margarine contains C18:1-isomer at a level of 11.66% in triglyceride fraction as shown in Fig. 9 analysed by our HPLC method. The content of *trans* isomers were dependent on the source of the fat, namely, marine oil or vegetable oil, and the degree of hydrogenation[30]. In general, products in Japan contain less *trans* isomers than those made in U.S.A. and Canada[21].

Unnatural fatty acids are known to be excreted into bile and milk. Several investigators have described C18:1-isomers excreted into human milk[32], and a good correlation was reported between their presenece in the milk and dietary margarine consumption[13,24].

For investigation of the metabolism of these isomers, bile was collected from three patients through a T-tube inserted into the common bile duct. Venolipid (500 ml) was infused for one hour, and bile samplings were collected before the administration, and at 30 minutes intervals for 24 hours, and after 48 and 72 hours. The C18:1-isomer was not detected in bile during 72 hours.

C18:1-isomers are orally consumed, but neither their accumulation in blood nor serious



		PL %	TG %
①	$C_{12:0}$	—	0.26
③	$C_{18:3}$	0.56	0.14
⑤	$C_{14:0}$	1.63	0.21
⑧	$C_{18:2}$	23.78	63.68
⑩	$C_{16:0}$	24.14	7.47
⑪	$C_{18:1}$	6.48	12.98
⑪′	$C_{18:1}$-isomers	1.56	11.66
⑫	$C_{18:0}$	38.48	3.59
⑬	unidentified		
⑭	longer chain fatty acids		

Fig. 9. Snow brand margarine

metabolic disturbances has been reported. Thus, it appears that C18:1-isomers can be converted to natural *cis*-isomers or excreted from the human body. There is a close correlationship between isomers in dietary fat and fatty acid composition in rat[11,19] and human[8,12] tissues, and their metabolism[29] and effects on the metabolism of other fatty acids in rats have been partly elucidated[20,27,28]. It has been reported recently that C18:1-isomers suppress prostaglandin synthesis[18] and aggravate EFA deficiency[15,35], and that they might be converted to prostaglandin precursors in the rat[33].

However, the effect of intravenously administered C18:1-isomer on serum fatty acid composition and bile in human subjects have not been reported. It still remains to be elucidated whether the isomer is metabolized[30] and excreted from the body or changed to natural *cis*-fatty acids and whether they affect physiological phenomenon in human subjects.

To elucidate the metabolism, our new HPLC method is also a valuable method for investigators. Moreover, its high accuracy with high sensitivity and easy operation will permit increased application of HPLC for fatty acid analysis in the near future.

Conclusion

Clinical applicability of our new HPLC method for fatty acid analysis was evaluated and usefulness of a fat emulsion for prevention of EFA deficiency was discussed by comparing fatty acid composition in serum phospholipid between two groups: twenty-four patients undergoing major abdominal surgery were divided into the Venolipid group (n=16), receiving the fat emulsion for seven days, and the fat-free group (n=8). Also a new parameter (*omega*-9/*omega*-6 ratio) to assess the EFA status was evaluated.

A) The clinical usefulness of the new HPLC was demonstrated.

1) Rapid and accurate determination of PUFA, especially EPA and DHA was possible.

2) Rapid assessment of EFA status was possible: Linoleic acid, arachidonic acid and eicosatrienoic acid contents, triene/tetraene ratio and *omega*-9/*omega*-6 ratio were determined rapidly. The *omega*-9/*omega*-6 was found to assess EFA status more sensitively than the t/t ratio.

3) C18:1-isomer was separated from natural *cis*-C18:1 (oleic acid). The isomer is present in the phospholipid of Venolipid. This is the first paper describing the intravenously administered C18:1-isomers. No serious side-effects were found.

4) Fatty acid transfer from the phospholipid to triglyceride did not take place, judging from the findings that no C18:1-isomer was detected in the latter fraction obtained after 7 days administration.

B) The usefulness of a fat emulsion was evaluated with respect to EFA status.

1) Venolipid is a useful fat emulsion for prevention of EFA deficiency, because it suppressed markedly *omega*-9 fatty acid synthesis, especially eicosatrienoic acid, in such a short-term trial of seven days.

2) An intravenously administered fat emulsion induced decreases in EPA and DHA in serum phospholipid in the early postoperative period.

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和文抄録

新 HPLC 脂肪酸分析法の臨床応用
(2) 脂肪乳剤が術後患者の血清脂肪酸構成に及ぼす影響
特に $C_{18:1}$ 異性体について

京都大学医学部外科学教室第2講座（指導：日笠頼則教授）
佐藤　友信，谷村　弘

外科領域においては，経静脈および経腸高カロリー栄養法の導入により術前・術後の栄養管理に飛躍的進歩がもたらされ，必須脂肪酸（EFA）欠乏症の治療と発症防止の重要性が特に認識され，経静脈的な脂肪乳剤の投与が欠かせないものとなった．

いわゆる EFA といわれるリノール酸やアラキドン酸の測定以外にも，従来無視されてきた EPA $C_{20:5}$, DHA $C_{22:6}$ など高級不飽和脂肪酸の測定にも著者らの開発した HPLC 法が極めて有用であることを確認し，その応用例の1つとして，経腸栄養剤に含まれる脂肪が血清リン脂質の EPA, DHA の体内代謝に影響を与えることを見出した．

今回，新しい脂肪乳剤 Venolipid® の静脈内投与が血清脂肪酸，とりわけ EPA, DHA, EFA に与える影響を検討し，著者らの提唱している ω-9/ω-6 比を指標として用いる EFA 状態の評価法の妥当性を検討した．

腹部外科術後症例24例において，術翌日より Venolipid 1日 500 ml 7日間，投与群（n＝16）と非投与群（無脂肪）（n＝8）の2群に分け，投与前，終了翌日（8日目），終了1週間後（15日目）に血清リン脂質分画の脂肪酸分析を施行し，以下の結論を得た．

1) EPA, DHA は，Venolipid 群ではいずれも8，15日目に減少したのに対し，無脂肪群では15日目にのみ減少した．

EPA, DHA は，脂肪の静脈内投与によっても，経腸投与時と同様に，かなりの影響を受けることを明らかにした

2) いわゆる EFA であるリノール酸とアラキドン酸は無脂肪群の15日目に減少した．

3) ω-9 系の $C_{20:3}$ は，無脂肪群で15日目に増加したのに反し，Venolipid 群では8日目に著明に減少（$p<0.01$）し，$C_{16:1}$, $C_{18:1}$ も減少した．

4) Venolipid 群では，triene/tetraene 比が8日目に0.24±0.02から0.17±0.02へ，ω-9/ω-6 比が0.74±0.05から0.47±0.02へといずれも低下した．一方，無脂肪群では，ω-9/ω-6 比が15日目に0.55±0.04から0.71±0.06へと増加した．

5) Venolipid のリン脂質は天然には存在しない $C_{18:1}$-isomer を35.29％含有していることを指摘し，その Venolipid の投与により，患者血清のリン脂質分画にも，投与終了翌日に 44.76±2.61 mg/dl 検出された．しかし，その1週間後，血清及び，胆汁には，もはや検出されなかった．

6) $C_{20:3}$ の著明な減少および，EFA 状態の指標としての ω-9/ω-6 比の改善効果から，EFA 欠乏状態の予防のためには，脂肪乳剤の投与が有効であることを確認した上に，EPA, DHA などの高級不飽和脂肪酸や $C_{18:1}$-isomer の体内代謝の解明に，著者らの開発した HPLC 脂肪酸分析が極めて有用であることを立証した．